oxidase or sphingomyelinase. In addition, the effective domain area explored by tracer particles increased post drug treatments, from 0.25 to 0.31 um^2. These observations suggest that the domain boundaries are affected by drugs leading to more permeable domains.

1783-Pos Board B675
Experimental Determination of the Forster Critical Distance
Tanya A. Baldwin, Gi-Ho Kim, Christopher Solis-Ocampo, Maria E. Moutsoglou, John M. Robinson.
South Dakota State University, Brookings, SD, USA.
Forster resonance energy transfer (FRET) can be used as a spectroscopic ruler to measure nanometer-scale distances. The recovery of inter-dye distance depends on a calibration factor known as the Forster critical distance (R_0). This distance is currently estimated based on measurements of the quantum yield of the donor dye, the overlap integral between the donor and acceptor dyes, and assumptions about the index of refraction and the relative orientation of the donor and acceptor dye molecules.
Here, we report a method to experimentally measure R_0, using B-DNA as a structural reference. Fifteen donor (Cy3)-labeled oligonucleotides were generated, by placing donor-labeled Thymidines at positions 11, 14, ⋯, 39. A single complementary strand was synthesized with acceptor (AlexaFluor647) at position 10. The strands were annealed, producing dsDNA consisting of a 30 base pair (bp) ruler with a 10 bp cap on each end. For each freely diffusing construct, the mean transfer efficiency (TE) was measured by single-pair FRET (sp-FRET) and ensemble (en-FRET). The TE's as a function of bp were fit to a reduced representation model of B-DNA that provided the absolute inter-dye distances. The reduced model was formulated based on an atomistic model of dye-labeled B-DNA, R_0 was recovered from the fit. We repeated our approach using three different donor/acceptor pairs, each with a different R_0.

1784-Pos Board B676
How can the Enhanced Sensitivity and Favourable Noise Characteristics Conferred by Electron Multiplication Improve Fluorescence-Guided Surgery?
Yoann Gosselin[1], Brian C. Wilson[2], Keith D. Paulsen[3], Kolbein Kolste[3], David W. Roberts[4], Pablo A. Valdés[5], Leblond Frederic[1].
[1]Polytechnique Montreal, Montreal, QC, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Thayer School of Engineering, Hanover, NH, USA, [4]Dartmouth Hitchcock Medical Center, Hanover, NH, USA, [5]Dartmouth Medical School, Hanover, NH, USA.
Biomarkers are traceable substances that can be used to monitor pathological molecular processes and in particular to guide the resection of tumors. A biomarker that is currently being used clinically is the protoporphyrin IX (PpIX), which has been demonstrated to preferentially accumulate in high-grade glioma following administration of 5-aminolevulinic acid. In the case of high-grade glioma, PpIX accumulates in a sufficient manner that it can be seen through the naked eye when exposed to blue light. However, higher sensitivity is needed to detect PpIX in other types of brain tumors where PpIX levels are lower or to achieve more complete resections. Higher sensitivity has been achieved using a fiberoptics point-probe allowing surgeons to detect PpIX with an unparalleled sensitivity *in vivo* during gliomas surgeries. However, point-by-point detection disrupts the neurosurgical workflow and so less disrupting wide-field imaging methods with high sensitivity and rapid image acquisition are desirable.
We investigate whether or not electron multiplication devices (EMCCD) can confer sensitivity and acquisition time advantages in fluorescence-guided neurosurgery when compared to scientific-grade charge-coupled devices (CCD). We present a demonstration of the sensitivity of PpIX detection that can be obtained with a new EMCCD camera that was developed by Nüvü Cameras. This device significantly reduces the noise generated during the read-out process (at least 10x) and presents a previously unreachable sensitivity in photon counting mode. Such major reduction in the noise threshold represents an opportunity to detect very faint levels of PpIX with smaller integration times than was previously achieved with CCDs. The goal of this study was to open the way to a less disruptive *in vivo* fluorescence detection technique to allow surgeon to perform more accurate resections on a more varied range of intracranial tumors

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Diffusion-Enhanced Luminescence Resonance Energy Transfer in the Cytoplasm of Live Bacterial Cells
Robert Renthal, Abhinaya Govindaraj, Nayanish D. Lokhande, Daniel Gonzalez, Aaron Cassill.
University of Texas at San Antonio, San Antonio, TX, USA.
We previously found that a 17-amino acid lanthanide-binding tag (LBT) expressed as a fusion with a cytoplasmic protein in *E. coli* takes up Tb^{3+} in live cells (Biochemistry 50, 6789, 2011). The protein is called DAL after its three domains: dihydrofolate reductase, ankyrin repeats, and LBT. We expressed DAL with a C-terminal 6X-His-tag to create a transition metal binding site for an acceptor of luminescence resonance energy transfer (LRET) from the Tb^{3+} donor on the LBT. Cu^{2+} added outside the cells appeared to be transported into the cytoplasm, where it quenched the luminescence of DAL-His-tag similarly to purified DAL-His-tag in free solution. When we expressed DAL lacking a His-tag, we also observed Cu^{2+} quenching of luminescence, and removal of the highly charged ankyrin domains did not alter Cu^{2+} quenching. The quenching mechanism is likely to be LRET, because Cu^{+}, which lacks spectral overlap with Tb^{3+}, does not quench DAL luminescence. We conclude that the energy transfer probably occurs by a diffusion-enchanced mechanism. In the small volume of a bacterial cell, freely diffusing donors and acceptors are likely to come in contact during the excited state lifetime of Tb^{3+} (2 msec). This suggests a possible method for measuring association-dissociation equilibria of large macromolecuar complexes in live bacterial cells. Protein monomers labeled with LRET acceptors (e.g. GFP or FlAsH-tags) can quench DAL donors by diffusional LRET. When associated into a few large assemblies, the acceptors will have much slower diffusion and their locations will no longer be distributed throughout the cytoplasm. We expect that after large assemblies form, diffusion-enhanced LRET will greatly decrease. We plan to test this on *E. coli* assemblies such as the fiber-forming enzyme CTP synthase, and the ethanolamine utilization microcompartment.

1786-Pos Board B678
Phosphatidylinositol 4-Kinase IIβ Associates with Clathrin Coated Vesicles in Living Cells as Revealed by Brightness Analysis
Jinhui Li[1], Barbara Barylko[2], Joachim D. Mueller[1], Joseph Albanesi[2], **Yan Chen**[1].
[1]University of Minnesota, Minneapolis, MN, USA, [2]UT Southwestern, Dallas, TX, USA.
Mammalian cells express two classes of phosphatidylinositol 4-kinase (PI4K), designated Types II and III, which phosphorylate phosphatidylinositol to generate PI4P. A number of studies indicate that these enzymes are important for Golgi trafficking and early as well as late stages of endocytosis. In this study, we focus on PI4KIIβ, a protein that is evenly distributed between membrane and soluble fractions and is believed to participate in stimulus-dependent phosphoinositide signaling. Using molecular brightness analysis, we found that EGFP-tagged PI4KIIβ exists as two distinct species in the cytoplasm, a soluble monomer and a high order complex enriched with multiple copies of PI4KIIβ. This observation is confirmed by autocorrelation analysis which identifies two species with distinct mobilities. We further demonstrate that the high order complex enriched with PI4KIIβ is sensitive to inhibition of palmitoylation, indicating that it is associated with membranes, very likely vesicles. Indeed, we show that the high order PI4KIIβ complex is sensitive to expression of dynamin 2-K44A, a dominant-negative inhibitor of endocytosis. We further directly detect that PI4KIIβ co-moves with clathrin light chain on vesicles using dual-color heterospecies partition analysis. This analysis allows us to isolate the co-mobile species in the presence of strong background contribution from the monomeric pool of PI4KIIβ. Our results strongly suggest that PI4KIIβ is involved in an early stage of endocytosis, and associated with clathrin-coated vesicles. Moreover, we establish molecular brightness as a powerful tool to characterize cellular cytosolic vesicles that are otherwise difficult to characterize by other techniques. This work is supported by the National Institutes of Health (R01 GM64589) and the National Science Foundation (PHY-0346782).

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Removing pH-Sensitivity and Improving Photostability of a Genetically-Encoded Chloride Sensor
Sheng Zhong, Dhasakumar Navaratnam, Joseph Santos-Sacchi.
Yale University School of Medicine, New Haven, CT, USA.
Chloride is the major anion in cells, and related to many diseases with disordered Cl^- regulation. For the non-invasive investigation on Cl^- flux across the cell membrane, YFP-H148Q and its derviates (Galietta, Haggie et al, FEBS Lett. 2001, 499(3), 220-4) were introduced as genetically encoded chloride indicators, including the CFP-YFP-based ratiometric Cl^- indicator Clomeleon(Kuner and Augustine, Neuron. 2000, 27(3), 447-59) and Cl-sensor (Markova, Mukhtarov et al, J Neurosci Methods. 2008, 170(1), 67-76). Neither the Cl^- sensitivity (Clomeleon) nor the pH-sensitivity (Cl-sensor) is satisfactory enough for accurate Cl^- measurements under physiological conditions. Additionally, the relatively poor photostability of YFP derviates hinders their